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14. ABSTRACT
The goal of the GWI consortium is to develop a better understanding of GWI and identify specific disease targets to find treatments that will address the cause of the disease. The consortium will integrate our clinical understanding of the disease process with basic research efforts using a novel mathematical model. The computational biology approach will enable the consortium to quickly identify targets of dysfunction and find treatments that will address the causes of the disease. The project will combine animal models of GWI with focus on the immune, cardiovascular and autonomic systems.

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INTRODUCTION

The underlying mechanisms of GWI remain unknown and treatment has been palliative, symptom-driven and physician-directed. The purpose of this multidisciplinary consortium project is to investigate animal GWI models with the goal of testing chemical treatments. The immune and autonomic biomarkers will be tested using a computational modeling approach allowing for a critical analysis and an accurate selection of test agents. The idea is to combine animal and human studies – a translational approach. Animal studies will be followed by clinical trials with agents thought to be most efficacious.

KEYWORDS

Autonomic Dysfunction
Computational Biology
Cytokines
Deregulated Balance
Diisopropyl Phosphorofluoridate
Drug Treatment
Electrocardiogram
Exercise Physiology
Gulf War Illness
Homeostasis
Molecular Targets
Mouse Model
Parasympathetic
Putative Therapeutics
Regulatory Network Configuration
Repurposed Drugs
Sarin
Stress Response
Sympathetic
Target Intervention
Therapeutic Interventions
Translational Human Clinical Trials
Translational Models

ACCOMPLISHMENTS

What were the major goals of the project?

	Timeline (Months)	Percentage Complete
Major Task 1: Setup the administrative structure required for the conduct of the animal and human studies		
Subtask 1: Prepare Regulatory Documents and Research Protocols for Study 1		
Prepare, submit and receive approval for animal protocols	1-4	100%
Refine experimental protocols via conduct of preliminary experiments.	4-12	80%
Refine eligibility criteria, exclusion criteria, screening protocol	3-12	100%
Complete prototype consent form & human subjects protocol	3-12	100%
Submit amendments, adverse events and protocol deviations as needed	As Needed	100%
Coordinate with Sites for annual IRB** report for continuing review	Annually	0%
Subtask 2: Establishment of administrative structure including coordinating center and database system		
Recruit, hire and train key personnel, students, staff and faculty	1-6	100%
Setup the coordinating center including database setup	1-12	90%
Setup administrative including committee appointments and scheduling of key review meeting	1-5	100%
Development of reporting procedures – minimum of updates every 6 months.	3-12	100%
Finalize consent form & human subjects protocol , receive approval	24-36	0%
Annual meeting with the consortium members and the external advisory committee – live and via internet	As scheduled	100%
Meetings in the DC region with DoD staff and representatives of the groups – twice per year	As scheduled	100%

	Timeline (Months)	Percentage Complete
Major Task 2: Refinement and enhancement of animal models for GWI.		
Sub task 1: Establish the model of autonomic dysfunction as a surrogate for GWI.		
Train staff and students in specialized surgical methods used to setup for monitoring autonomic function.	Begin 3 and continue	100%
Test cholinergic toxins in mice with examination of peripheral autonomic and cardiac function – predict long term deficits	4-15	30%
Employ spectral analytical methods for examination of sympathetic and parasympathetic balance	Begin 4 continue	30%
Conduct wheel running acute and chronic exercise tests to simulate the exercise model in humans	4-12	25%
Combine tests of acute and chronic exercise in the GWI chemical toxin model, providing an excellent preclinical comparison.	12 continue	25%
Submit animal protocol amendments as required	As needed	100%
Measure immune biomarkers in the autonomic dysfunction model, compare to measures of adrenal function	24 continue	0%
Extend preliminary analysis to transcriptional level. Filter and normalize data using accepted best practices and perform traditional analysis of expression profiles at the level of individual genes	6-18 continue	10%
Successful use of data coordination/statistical analysis center bringing together large amounts of data from multiple systems	5 continue	5%
Subtask 2: Establish the model of DFP/cort as a surrogate for GWI.		
Train staff and students in conduct of model	Begin 3 and continue	75%
Test cholinergic toxins in mice with examination of immune markers in brain and periphery	4-15	20%
Employ analytical methods for examination of immunological balance	Begin 4 -16	10%
Establish the minimum levels of corticosterone required to maintained a heightened pro-inflammatory response to the sarin surrogate, DFP	4-12	0%
Evaluate stress regimens to establish protocols required to exacerbate proinflammatory response to sarin surrogate, DFP	5-15	10%
Submit animal protocol amendments as required	As needed	100%

	Timeline (Months)	Percentage Complete
Subtask 3: Characterize the molecular and cellular phenotypes of GWI mouse models with the idea of using them to test treatments.		
Use transcriptional analysis to study the immunological basis for the brain and blood changes in the GWI models	12 -24	25%
Use bioinformatic method to estimate pathway activation from gene expression and conduct comparisons between mouse and humans.	6-18	25%
Use molecular modeling to identify and develop networks of expression allowing for robust comparisons between GWI and animal models. Test under baseline and stimulated (stress hormones or exercise)	12-30	40%
Major Task 3: Identification of Illness specific networks with focus on human and mouse comparisons		
Subtask 1: Conduct network analysis for humans and animal models		
Apply biological modeling techniques to pathway activation computed in task 2 sub 3 to render pathway networks	6-12	2%
Integrate with other levels of biology then identify and compare functional modules at various resolutions across groups	12 -18	15%
Conduct detailed analysis of network topology applying measures of network structure and information flow to identify critical information-processing modules	12-24	20%
Conduct an analysis of the alternate steady states available to the regulatory networks identified in human and mouse models.	12-30	40%
Inform pathway-specific genomic panel based on the key network regulatory pathways	12-30	50%
Major Task4: Large-scale simulation of treatment.		
Subtask 1: Conduct in silico sensitivity analysis and rank candidate target nodes		
Use simulation experiments to assess and rank the impact of introducing an in silico equivalent standardized treatment pulse or pulse train at each node in turn throughout the model network	18-30	40%
Rank the candidate target nodes in terms of their relative contribution to shifting the structure of the network recovered under treatment and the network presented in healthy control subjects	18-30	15%

	Timeline (Months)	Percentage Complete
Major Task 5: Define and deploy large-scale optimization.		
Subtask 1: Evaluate and select the best global search algorithm for targeting intervention possibilities		
Review latest developments in evolutionary programming techniques as well as hybrid gradient-based techniques to determine the most suitable search algorithm. Acquire or develop code and deploy.	12-18	30%
Configure simulation-based optimization scheme that evaluates the fitness of candidate interventions by repeatedly launching short network simulation runs in search of the most robust treatment course	18-24	40%
Major Task 6: Identify candidate treatment courses for GWI		
Subtask 1: Using task 5 launch optimization runs from multiple initial conditions of endocrine-immune status		
Identify and describe mathematically the immune and endocrine descriptors that can be effectively and safely changed and over what range they may be changed.	24-30	2%
Using drug databases and bioinformatic techniques identify drugs currently available for repurposing to treat GWI	12-30	25%
Search for novel treatment courses. Launch repeated searches for optimal treatments using the set of candidate cytokine, hormone/autonomic and immune markers isolated in task 5	24-36	10%
Major Task 7: Identify candidate treatment courses for GWI		
Subtask 1: Select and test pharmacological therapies on basis of data from computational models in animals		
Use previous data to select best animal models based on immunological and autonomic biomarkers	24-36	40%
Develop computer/mathematical paradigms for evaluation of treatment strategies	12-30	40%
Develop pilot clinical trials on basis of animal studies	24-36	0%

	Timeline (Months)	Percentage Complete
Major Task8: Verify treatment effectiveness in human subjects		
Subtask 1: Studies of treatment effectiveness in humans		
Design assessment platform for use in human translational studies using the RedCAP platform as a foundation	18-24	90%
Complete the IRB process for selected study drugs, using the Miami VAMC IRB with OCMR review.	24-30	0%
Recruit and perform assessments of GWI subjects on intervention(s) in the phase 1 translational studies.	30-40	0%
Evaluate change in network interactions from interventions suggested Study 3 and 4. Inform the model with the human study data and refine as necessary	32-48	0%

What was accomplished under these goals?

- CDC ACUC Protocol and ACURO protocols were renewed. **(Task 1; Subtask1)**
- VA Hospital Miami ACUC protocol (Chemical Toxicity and Cardiovascular Function (5841.02) and ACURO were renewed. **(Task 1; Subtask1)**
- Dr. Steele from Baylor continued to act primarily in a consulting role for the consortium, working with Drs. Morris and Klimas on study planning, particularly in relation to studies involving clinical assessments, interventions, and evaluation of Gulf War illness treatment outcomes. **(Task 1; Subtask 1)**
- Dr. Morris hired Dr. Jacqueline Machi, PhD as a Research Assistant to work full time for the GWI consortium. **(Task 1; Subtask 2)**
- Felipe Conte and Rodrigo Schmidt are PhD students who have committed to one year on the project. They will be supported on the Brazil interchange program. **(Task 1; Subtask 2)**
- Dr. Diana Hernandez has been hired as Dr. Morris' Research Associate and will be conducting the animal test along with Dr. Luis Salgueiro of the Miami VA. **(Task 1; Subtask 2)**
- Recruited new volunteer research assistant Ms. Mariam Viquar, Doctor of Osteopathic Medicine (DOM) class of 2016, to continue genomic analysis of available human data under the supervision of Dr. Craddock. **(Task 1; Subtask 2)**
- Participated in EAB teleconference meeting on April 14, 2015. **(Task 1; Subtask 2)**
- Had monthly executive board meetings to discuss the study. **(Task 1; Subtask 2)**
- Had monthly investigators meetings to discuss the study. **(Task 1; Subtask 2)**
- Currently having weekly meetings at the Miami VA to discuss the animal studies in Dr. Morris' lab space as well as the lab tests being run at Dr. Fletcher's immunology lab. **(Task 1; Subtask 2)**
- Had our annual consortium member meeting on September 25, 2015 at the NSU Guy

Harvey Oceanographic Center. Discussed modeling targets, possible repurposed drug treatments that fit the models, the progress of the animal studies at the CDC and at the Miami VA, preliminary data, upcoming EAB meeting, future collaboration between Dr. Broderick's team and the team from Southwest Research Institute to "fine tune" the drug treatments, and possible timelines for getting the human subject testing started. **(Task 1; Subtask 2)**

- On February 12, 2015, Dr. Broderick submitted 1st detailed report in follow up of Fort Detrick EAB meeting (September 2014). **(Task 1; Subtask 2)**
- On March 4, 2015, Dr, Broderick submitted a 2nd interim report to address additional questions asked by Dr. Reifman on March 2, 2015. **(Task 1; Subtask 2)**
- Dr. Broderick met with EAB members Dr. Reifman and Dr. Lidie via teleconference orchestrated by Mr. Chaney on March 4, 2015 to review contents of both interim reports. Meeting closed with a request for a third interim report to cast numerical protocols in the context of clear research questions for each study. **(Task 1; Subtask 2)**
- April 11, 2015, Dr. Broderick submitted 3rd detailed interim report in follow up of March 4 teleconference. **(Task 1; Subtask 2)**
- Dr. Broderick presented refined protocol to EAB in semi-annual meeting, April 14, 2015. **(Task 1; Subtask 2)** This numerical protocol formally addressed:
    - Loss of signal and statistical power due to genetic homogeneity of mice and small group size (n=5) through concatenation of treated and untreated groups,
    - Bias effects of outliers and supports the reconstruction of time courses using repeated sub-sampling and a unanimous consensus scheme across all networks (each of which applied a FDR<0.05 significance threshold)
- Dr. Klimas has developed a phase 1 study design. We have the primary and secondary outcome variables identified, and have the RedCap platform in place, the instruments are being uploaded to this web based platform for Beta testing in the month of August. We will be ready to start the clinical trial when the animal modeling work's preliminary analyses become available in the winter of 2015/16 (with several months required for IRB and DoD human safety reviews). We will be underway no later than end of FY3 of the consortia study. **(Task 1; Subtask 2)**
- Measure immune biomarkers in the autonomic dysfunction model, compare to measures of adrenal function. Prior summer students Gaytri Patel and Trevor Barker prepared and submitted abstracts to 2 international conferences: (i) the Anxiety and Depression Association of America Annual Conference (ADAA) held in Miami in April 2015 and (ii) American Psychological Association Annual Conference (APA) held in Toronto in June 2015. Additionally, Ms. Patel and Mr. Barker submitted abstracts to the Miami VA Health Center for the National VA Research Week. These abstracts are based on their earlier analysis of differences in symptom profiles and patterns of immune marker co-expression in clusters of GWI subjects defined on the basis of Davidson Trauma score profiles. All abstracts were accepted as poster presentations. In parallel with this work graduate student Tory Toole and data control specialist Mark Rice also completed and submitted abstracts to the ADAA, and APA as well as the Association for Psychological Science (APS) annual convention. These were based on their study of regulatory control and how this might inadvertently promote persistence

of symptoms. Once again all abstracts were accepted and presented as posters. **(Task 2; Subtask 1)**

- *Extend preliminary analysis to transcriptional level*. A new volunteer research assistant, Ms. Mariam Viquar continued work started by summer 2014 intern Mr. Samuel Thomas. This work consisted of a confirmatory survey of transcription factor activation across exercise in human subjects with a focus on neuro-inflammatory processes. Ms. Viquar focused on completing the pathway analysis and assisted in preparing a draft report of the results. **(Task 2; Subtask 1)**
- *Animal work informing the human model*: Analysis of differences in symptom profiles and patterns of immune marker co-expression in clusters of GWI subjects defined on the basis of Davidson Trauma score profiles **(Task 2; Subtask 1)** produced a high trauma and a low trauma group. Blood cytokine profiles obtained in mouse at 21 days were compared to human GWI subjects under exercise challenge. Early results suggest that mouse exposure models should be evaluated against trauma-delimited sub-groups of GWI subjects instead of a single mixed population.
- GWI Trauma clusters differed significantly in proximity to the mouse signatures with the latter aligning much more readily with the high trauma group. Of these, the mouse signatures involving Cort (i.e. Cort+LPS and Cort+DFP+LPS) were statistically similar at peak effort (VO2max) to mouse response in blood at 6 hours post LPS challenge. **(Task 2; Subtask 1)**
- Network analysis of the co-expression patterns linking the 12 blood markers increased the resolution further. Our first results show that co-expression patterns in LPS response involving Cort priming are statistically proximal to the human GWI signature in blood. Comparison with networks obtained under other exposure conditions shows that i) networks involving Cort priming are more proximal to human co-expression networks and ii) that of these the network involving Cort+DFP+LPS is statistically distinguishable from Cort+LPS and closer to the human GWI co-expression pattern. **(Task 2; Subtask 1)**
- Finished setting up the cardiovascular/autonomic lab at the VA (Dr. Morris). This included purchase of equipment and animal testing. Dr. Machi and Dr. Salgueiro (from VA sub contract) have had training sessions with the company scientists and have the skills required for conducting long term experiments. Work is in progress to test the telemetry system. **(Task 2; Subtask 1)**
- At the CDC, 15d study - all dosing complete, samples taken and are being analyzed and paper write ups are in progress; 21 d study - all dosing complete, samples taken and are being analyzed and paper write ups are in progress; 90d study-all dosing has occurred, samples taken and are being analyzed and paper write ups are in progress. **(Task 2; Subtask 1)**
- ALDH1L1 mice used for pilot BAC-TRAP experiment with GWI experimental conditions at the CDC. **(Task 2; Subtask 1)**
- CX3CR1 -/- mice have been bred at the CDC and will be available for dosing and preliminary data collection. **(Task 2; Subtask 1)**
- Serum samples were shipped from CDC to NOVA group at 21 days and 90 days post mouse exposure to GWI producing conditions – July 2015 and September 2015. **(Task 2; Subtask 1)**
- Collaborations are being established with Dr. Eric Johnson from the Institute of

chemical Defense (USAMRICD). They will be responsible for dosing the mice with Sarin. The treated mice will be transferred to the Miami VA animal labs for experiments on autonomic function. We are awaiting approval of the CRADA from the USAMRAA/CDMRP. **(Task 2; Subtask 1)**

- A key part of the GWI consortium project is to compare results in mice and humans in terms of the response to exercise. Table 1 and Figures 1-3 (see appendices) show the autonomic response to a chronic wheel exercise paradigm. Using the Echo system we were able to quantify the autonomic pattern. This is a new and provocative method which can be applied to human studies. **(Task 2; Subtask 1)**
- The cardiac autonomic lab directed by Drs. Morris and Salgueiro at the Miami VA is fully operational in terms of methods, active animal protocols and initial experiments. Critical methods for the in vivo studies are echo cardiography (heart function), ECG (heart rhythms) and autonomic function (taken from high frequency ECG recording). **(Task 2; Subtask 1)**
- Collected ECG data using a Vevo 1100® Echo machine. The mice were kept under anesthesia (Isoflurane, 2 – 2.5% on 0.8l/min of O2 and attached to a board with ECG sensors. The electrical activity was captured and the program used derivations to build full wave. We used a sample rate of 8000Hz to capture the natural frequency for 3 minutes. The data was exported to a csv file and opened with Windaq® Software (DATAQ Instruments). We use an algorithm to detect R peaks and create a time series file containing all R to R intervals (RRI). This tachogram was used to calculate heart rate variability by Cardioseries® program. The time domain parameters were calculated by RRI mean, mean standard deviation and standard deviation of consecutive RRI (RMSSD). The frequency domain was performed by fast Fourier transform (FFT) whit 10 Hz of interpolation frequency. We use periods of 512 beats, 50% of overlap to run the calculation. The very low frequency (VLF) was not used (VLF<0.1Hz). Sympathetic modulation was considered as low frequency (LF) band (0.1<LF<1.0 Hz). The parasympathetic modulation was associated with the high frequency (HF) band (1.0<HF<4.0 Hz). Table 1 (see appendices) gives a list of parameters used to evaluate autonomic balance. **(Task 2; Subtask 1)**
- Termination of exercise autonomic study. Mice (n = 10 group) were exposed to daily wheel running for 1 hr. After 8 weeks they will be tested with DFP or Sarin. Final measures will focus on immunological changes in brain and heart. **(Task 2; Subtask 1)**
- All of the personnel at the VA animal lab have been trained to use all the equipment for the study (electrocardiogram machine, mass spectrometer, etc.) as well as the procedures for the animal protocols (surgical methods, dosing, etc.). **(Task 2; Subtasks 1 and 2)**
- Set up experimental protocol to compare the short term brain immunological response to sarin and DFP – 8 hr. time point with n of 10 mice per group (3 groups). **(Task 2; Subtask 2)**
- Preclinical treatment – mouse exposure to GWI has occurred and tissue has been collected and data processed for minocycline at early (7d) time point at the CDC. We also have mouse tissue collected for preliminary minocycline treatment at 90 days post LPS exposure and prior to the LPS challenge. Samples are scheduled to be processed October 2015. **(Task 2; Subtask 2)**
- Ms. Lindsey Russell completed her revised analysis of cytokine profiles in mouse brain

and blood emerging in response to corticosterone (Cort) pretreatment and subsequent challenge with lipopolysaccharide (LPS). This work was supervised by Dr. Broderick, with the help of Drs. Craddock, Miller and O'Callaghan. A complete manuscript is currently under internal review by members of the authorship team. **(Task 2; Subtask 2)**

- CDC gained results on cytokine expression in the cortex at 21, 90 and 97 days following initial GW relevant exposure (4 days of Cort in drinking water, and single injection of DFP on day 5) that was followed up with administration of Cort in drinking water for 4 days every other week until either 21, 90, or 97 days. On this last day, mice were challenged with an inflammagen (LPS). Results in Figures 4 and 5 (see appendices). **(Task 2; Subtask 2)**
- CDC conducted an experiment with minocycline as an anti-inflammatory treatment. Mice were exposed to our initial GW theater-like conditions (4 days of Cort in drinking water, DFP on day 5) and then given an inflammagen challenge (LPS) 48 h later (on day 7). Mice received either saline or minocycline one hour prior to LPS exposure. Results in Figure 6 (see appendices). **(Task 2; Subtask 2)**
- Lead by Dr. Morris and Dr. Klimas, Rodrigo Schmidt has setup methods to do autonomic measurements in humans exposed to exercise. **(Task 2; Subtask 3)**
- Using the updated protocol, conducted network analysis of corticosterone potentiation of immune and neuro-immune response in mice **(Task 2; Subtask 3)**. Specifically, performed analysis of association network properties linking blood-borne cytokine concentrations and expression of immune messenger RNA in hippocampus and cortex in mice for:
    - *Initial exposure to agents*. Results show corticosterone dramatically increases the size and persistence of coordinated (networked) immune response to both LPS and DFP across 2, 6 and 12 hours post-exposure at 5 days.
        - LPS response: Analysis of network response to LPS and Cort potentiated LPS present with central mediators mainly in blood (followed by hippocampus). Specifically, blood-borne MIP-2 emerges as central mediator early in Cort+LPS response with KC playing a central role late in the response to LPS.
        - DFP response: Networked response to DFP exposure alone show cortex CCL2 as a central mediator. In the case of Cort-potentiated response to DFP, hippocampus GFAP and cortex TNFa emerge as early mediators. In both cases the responses on blood are diffuse and no clear central mediators arise in the periphery.
        - Propagation effects. In looking across all 3 time points i.e. 2, 6 and 12 hours post-exposure, we conducted a network propagation analysis to highlight small changes in markers at 2 hours that would initiate larger immune response cascades. We found that small changes in blood-borne KC prompt the emergence of an immune network in hippocampus at 6 hours that eventually recruits a response in cortex at 12 hours in response to LPS exposure. When LPS is potentiated by Cort exposure the KC-prompted network is

larger at 6 hours, involves all 3 compartments but dissipates before 12 hours.
        - Repeating this propagation analysis for all 26 markers we found:
            - Important trigger effects for several markers in blood and brain in response to LPS. When exposed to Cort and LPS, the primary initiators were primarily blood-borne IL-1b, IL-6 and KC.
            - Response triggers for DFP, Cort+DFP were mainly in brain, namely hippocampus GFAP and TNF, as well as cortex IL-6.
    - *Subsequent medium-term challenge with corticosterone and LPS (21 days)*. The basic premise for the mouse model is one of an exacerbated response to LPS, specifically in brain, following an earlier exposure to DFP and associated priming of the neuro-immune system. In our analysis of the blood cytokines and brain transcripts in mice exposed at 21 days to cortisol and LPS following different combinations of prior exposure. In our analysis of priming with Cort+LPS and Cort +DFP+LPS we found:
        - Cort potentiated responses to LPS with and without prior DFP priming both show significant fold-change in brain IL-1b, TNFa and CCL2 versus priming in the absence of Cort (DFP+LPS or LPS alone). Responses in blood were dominated by FC increases in IL-6 and KC under the same conditions.
        - Though association networks linking brain transcripts and blood markers in mouse with and without DFP priming shared a similar number of connections on average, these connections were distributed very differently and gave rise to more tightly connected local neighborhoods in mice with DFP priming.
        - Networks describing response to 21-day Cort+LPS challenge showed significantly different co-expression patterns when primed with Cort and DFP at 5 days (graph edit distance $p<0.001$). Central mediators of immune signaling were less abundant in DFP primed brains suggesting a more diffuse or shared signaling processing. Conversely DFP priming gave rise to several central mediators; IL-1 and MIP2 are central to DFP primed signaling in this case only.
    - Longer-term persistence of effects (90 days). We continue to gather data on this late course exposure.
- Dr. Broderick met with Drs. O'Callaghan and Miller at the CDC NIOSH labs in Morgantown, WV from February 24-27, 2015. He performed site visit and review of animal protocols for Study 2. Also reviewed exiting preliminary data and discussed numerical analysis of this data with laboratory research staff. In addition, he delivered a talk to NIOSH leadership and staff. **(Task 3; Subtask 1)**
- Dr. Broderick visited Dr. McGowan (PI - W81XWH-14-1-0550) at University of Toronto at Scarborough from March 30-31, 2015. Reviewed animal protocols for sister CDMRP project W81XWH-14-1-0550 with laboratory staff in order to align the analysis of histone modification and DNA methylation in exposed animals with data collected under GWIRC studies 1 and 2. **(Task 3; Subtask 1)**

- A subcommittee has been established to select the drugs for the animal experiments. Their focus is on translatable strategies for the human model. The subcommittee consists of Dr. Klimas, Dr. Craddock, Dr. Broderick, and Dr. Gutierrez. (**Task 6; Subtask 1)**
- Dr. Klimas has designed an assessment platform using REDCap for the human translational studies. It will be complete once a study drug has been identified and any safety related assessments are added. **(Task 8; Subtask 1)**
- Although not listed in the SOW goals above, one of the major goals of the GWI consortium is to promote and forward the momentum of the field using GWIC infrastructure. To this, we have 6 grant applications from Nova that will be submitted in October to the DoD that ask for collaboration or use material/work product of our consortia. We also have 4 other grant applications going in from other sites that have one or more consortia investigator listed as co-investigators. Dr. Klimas was awarded a DoD grant for a phase I/II clinical trial that used computations from our consortia. It is titled “Testing the Model: A Phase I/II Randomized Double Blind Placebo Control Trial of Therapeutics: Liposomal Glutathione and Curcumin.” She was also awarded a VA Merit grant titled “A Translational Medicine Approach to GWI: From Cells to Therapy.”
- Also to promote and forward the momentum of the field, this year we have published 2 papers integrating the GWIRC with concurrent efforts funded under affiliated project GW093042 (Broderick, PI) and its ongoing expansion award GW140142 (Broderick, PI). In the first paper, Cradock et al., 2015a, we report the results of a first computer assisted design for an idealized treatment course in GWI. This model-based optimization predicts that the two-phase delivery of an anti-inflammatory intervention followed by a glucocorticoid receptor blockade delivers the highest probability of remission under idealized conditions. Work is ongoing under new expansion award GW140142 to increase model fidelity. In the second paper, Craddock et al., 2015b, we report the results of a genomic analysis directed at identifying currently available drugs that might target components of GWI that are shared by other illnesses. This work consists of a bio-informatic analysis linking gene expression profiling data collected in human GWI subjects with known drug-gene interactions documented in pharmaco-genomic databases. This work exemplifies how the GWRIC serves as an integrative framework that effectively brings together elements form multiple projects in a highly cohesive and focused manner.
    - Craddock TJ, Del Rosario RR, Rice M, Zysman JP, Fletcher MA, Klimas NG, Broderick G. Achieving Remission in Gulf War Illness: A Simulation-Based Approach to Treatment Design. PLoS One. 2015a Jul 20;10(7):e0132774. doi: 10.1371/journal.pone.0132774. eCollection 2015.
    - Craddock TJ, Harvey JM, Nathanson L, Barnes ZM, Klimas NG, Fletcher MA, Broderick G. Using gene expression signatures to identify novel treatment strategies in gulf war illness. BMC Med Genomics. 2015b Jul 9;8:36. doi: 10.1186/s12920-015-0111-3.

What opportunities for training and professional development has the project provided?

- All of the personnel at the VA animal lab have been trained to use all the equipment for the study (electrocardiogram machine, mass spectrometer, etc.) as well as the procedures for the animal protocols. Faculty, staff, and students are encouraged to present their work at local and national meetings. They are also continuing opportunities for training.

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

- We will begin IRB and DoD approvals for human subjects in year 3 based on predictions from the animal studies.
- Once we have the CRADA in place, we can begin sarin mice experiments.
- Dr Broderick will be visiting the CDC again to talk future data analysis with new time points.
- CDC will be comparing 4 vs. 7 day initial Cort exposures and which yield the best outcomes for GWI phenotype.
- CDC will be beginning animals on GWI condition exposure this fall, so treatment testing can begin 90 days from now in GWI relevant model.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS

Changes in approach and reasons for change

MRI Global was originally going to supply the sarin treated mice. Dr. Morris decided to have the USAMRICD supply them since the cost would be lower and we would be able to use more animals.

Actual or anticipated problems or delays and actions or plans to resolve them

- We are having difficulty in obtaining the CX3CR1mice to be evaluated in our GWI dosing protocol. This was due to a NIH budgeting problem that caused discontinuation of the availability of this line from Taconic, the supplier with which we obtained mice to generate preliminary data. We now are putting an MTA together to obtain these mice directly from NIH and will set up a breeding line in house at CDC.
- There was a delay in getting the CDC funds through interagency agreement which slowed down the initial progress of the animal studies; effectively, Oct 1, 2014 was start of the CDC work. The studies are now proceeding as planned. The CDC site was having some difficulty in obtaining the CX3CR1mice to be evaluated in their GWI dosing protocol. This was due to a NIH budgeting problem that caused discontinuation of the availability of this line from Taconic, the supplier with which they obtained mice to generate preliminary data. Dr. O'Callaghan has put a transfer agreement together to obtain these mice directly from NIH and has set up a breeding line in-house at CDC to ensure supply of these animals for his planned studies. Breeding colony is being established in-house at CDC at no cost to DoD.
- CDC protocol is still under post-approval monitoring and veterinarian has decided no more animals may be exposed to DFP until many palliative care studies have been conducted to see if any care conditions reduce high mortality rate seen following exposure to DFP alone. CDC is continuing to try to find a resolution with the AV as quickly and efficiently as possible.
- We are experiencing delays with establishing the CRADA between the Miami VA and the USAMRICD. We are working with Jennifer Shankle, Grants Specialists USAMRAA, to get this resolved.
- There were delays with the Biosafety Committee at the Miami VA in regards to using DFP. After meeting many times, answering all their questions, and addressing their concerns, the use of DFP at the Miami VA was approved.

Changes that had a significant impact on expenditures

- Due to delays, expenditures are significantly less than what they should be. We are on an accelerated timeline and should be back on track with expenditures and research by the end of year 3. An amendment was made to the grant delaying year 3 funding until all the IRB approvals and DoD approvals are received for the clinical trial. We would like to point out that the protocol, as funded, did not intend to start the clinical trial until year 4. In year 3, there would be considerable animal and computational work as a therapy is selected and then we would submit protocols to the IRB, etc. to initiate the clinical trials in year 4.

- Since we have been delayed, we do not have enough data to present at the GWI Seminar and Program Evaluation that was slated for year 2. Because of this, we have decided to postpone it to year 3.

Significant changes in use or care of human subjects
Nothing to Report

Significant changes in use or care of vertebrate animals
Nothing to Report

Significant changes in use of biohazards and/or select agents
Nothing to Report

PRODUCTS

Publications, conference papers, and presentations

Journal publications.

- O'Callaghan JP, Kelly KA, Locker AR, Miller DB, Lasley SM. Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of Gulf War Illness. J Neurochem. 2015 Mar 5. doi:

Books or other non-periodical, one-time publications.

Other Publications, conference papers, and presentations.

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products
Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Mariana Morris, PhD
Project Role:	PI
Research Identifier:	eCommons: mariana
Nearest person month worked:	3
Contribution to Project:	Overseeing the entire research project. Established the animal protocols and in charge of the animal research. Oversees hiring of all personnel.
Funding Support:	NIH

Name:	Gordon Broderick, PhD
Project Role:	Co-Director
Research Identifier:	eCommons: gbroderick
Nearest person month worked:	3
Contribution to Project:	Head of computational biology. Has worked on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	NIH, VA, DoD

Name:	Travis Craddock, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: TRAVISCRADDOCK
Nearest person month worked:	3
Contribution to Project:	Has worked on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	NIH, CFIDS Association of America, Nova Southeastern University PFRDG

Name:	Nancy Klimas, MD
Project Role:	Co-Director
Research Identifier:	eCommons: nklimas
Nearest person month worked:	1
Contribution to Project:	Head of clinical sciences. Reviewed modeling from the computational biology team in regards to human subjects to help establish protocols.
Funding Support:	NIH, VA, CDC, DoD

Name:	Mary Ann Fletcher, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: mfletche
Nearest person month worked:	2
Contribution to Project:	Director of the immunology core.
Funding Support:	NIH, VA

Name:	James Blount
Project Role:	Administrative Coordinator
Research Identifier:	None
Nearest person month worked:	11
Contribution to Project:	Monitored budget, maintained meeting schedules, prepared quarterly and annual reports, assisted in establishing sub-awards, and other duties associated with administration of the award.
Funding Support:	None

Name:	Ana Del Alamo
Project Role:	Research Associate
Research Identifier:	None
Nearest person month worked:	4
Contribution to Project:	Assisted Dr. Klimas in in her work concerning the human subject protocols.
Funding Support:	None

Name:	Diana Hernandez, PhD
Project Role:	Research Associate
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Jacqueline Machi, PhD
Project Role:	Research Assistant
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Mark Rice
Project Role:	Data Control Specialist
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	In charge of the data analysis and has assisted on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	None

Name:	Jonathan Toole
Project Role:	Research Assistant
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Has assisted on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	None

Name:	Filipe Conti
Project Role:	Visiting Scholar/Graduate Student
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Active in animal experiments
Funding Support:	CAPES (Brazilian government agency)

Name:	Rodrigo Schmidt
Project Role:	Visiting Scholar/Graduate Student
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Has worked on the models to compare mouse to human.
Funding Support:	CAPES (Brazilian government agency)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
No

What other organizations were involved as partners?

Name:	Centers for Disease Control and Prevention National Institute for Occupational Safety and Health
Location:	1095 Willowdale Road Morgantown, WV 26505
Contribution:	Chemical toxicology project collaboration
Financial:	None
In-kind Support:	None
Facilities:	None
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None

Name:	Southwest Research Institute
Location:	5220 Culebra Road, PO Drawer 28510 San Antonio, TX 78228
Contribution:	Assisting on drug choices to test in animals and humans.
Financial:	None
In-kind Support:	None
Facilities:	None
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None

Name:	South Florida VA Foundation for Research & Education Inc.
Location:	1201 NW 16th Street, Room #2A103 Miami, FL 33125
Contribution:	Providing subjects and space for human trials in future. Help with establishing human protocols.
Financial:	None
In-kind Support:	None
Facilities:	Project staff uses the partner's facilities for project activities.
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other's facilities. Dr. Klimas and Dr. Fletcher are on staff at Nova Southeastern University and the Miami VA.
Other:	None

Name:	South Florida VA Foundation for Research & Education Inc. – Animal Facility
Location:	1201 NW 16th Street, Room #2A102 Miami, FL 33125
Contribution:	
Financial:	None

In-kind Support:	None
Facilities:	Project staff uses the partner's facilities for project activities.
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other's facilities. Dr. Morris is on staff at Nova Southeastern University and the Miami VA.
Other:	None

SPECIAL REPORTING REQUIREMENTS

Collaborative Awards:
Nothing to Report

Quad Charts: See next page.

Understanding Gulf War Illness: An Integrative Modeling Approach

Award Number: GW120045 / W81XWH-13-2-0085

PI: Dr. Mariana Morris **Org:** Nova Southeastern University **Award Amount:** $4,102,527

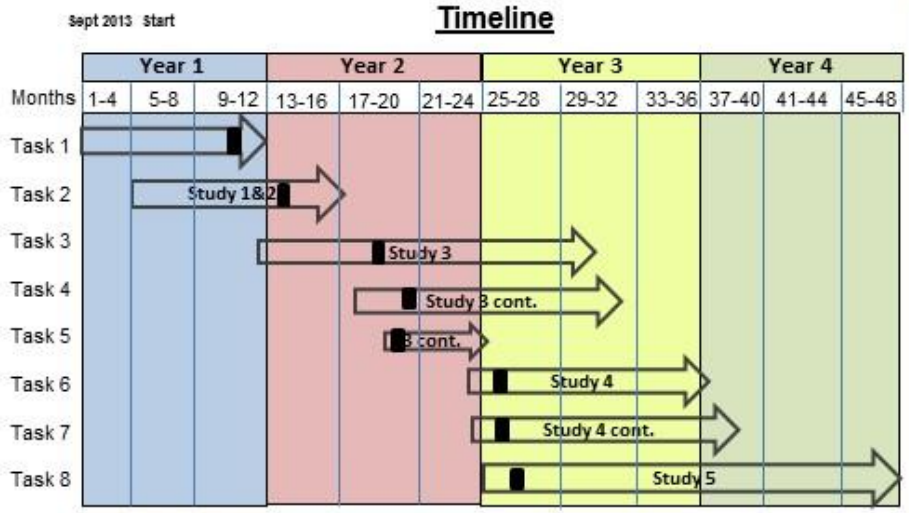
Approach To develop a translational model of GWI for rapid identification of molecular targets and prediction of effective therapeutic interventions. The effectiveness of candidate treatment in terms of system abatement and recovery of regulatory network configuration will be assessed in GWI subjects in phase 1 translational studies.

- ***Study 1:*** Characterize the autonomic neural/adrenal dysfunction in a mouse model of GWI using validation and direction from computational biology (**Task 2**).
- ***Study 2:*** Characterize the molecular and cellular phenotype of GWI in a mouse model to evaluate the role of stress response in persistence of the illness (**Task 2**).
- ***Study 3:*** Integrate human (previously completed) and animal studies using computational biology to identify mediators of deregulated balance and test putative therapeutics (**Task 3-5**)
- ***Study 4:*** Evaluate therapeutics suggested by computational model in GWI animal models. Two or three most favorable will move on to human testing (**Task 6-7**).
- ***Study 5:*** Perform translational human clinical trials to evaluate homeostasis "reset" as well as preliminary safety and efficacy (**Task 8**).

Accomplishments to date

1- CDC IACUC approval obtained and protocol has been approved by DoD ACURO
2- Miami VA animal protocol approved by VA IACUC. Approved by DoD ACURO
3- Developed methods for measurement of ECG in conscious mice.
4- Developed NanoString method for use with immunological markers.
5- Received approval for DFP use at Miami VA.
6- O'Callaghan et al. published Corticosterone Primes the Neuroinflammatory Response to DFP in Mice: Potential Animal Model of Gulf War Illness. J. Neurochem.
7- Designed assessment platform for use in human studies in REDCap.

Timeline



Goals/Milestones

FY13 Goal – Administrative structure for animal/human studies (Task 1)

- ☑ Kick-off meetings with GWIRP staff and study PIs
- ☑ Protocol preparation and initiation of approvals for animal/human use
- ☑ Coordinating center database set-up

FY14 Goal – Studies 1- 3 - Refinement and enhancement of models for GWI

- ☑ Establish model of autonomic dysfunction as a surrogate for GWI **(Task 2)**
- ☑ Identification of Illness specific networks with focus on human and mouse comparisons **(Task 3)**
- ☐ Large-scale simulation of treatment. **(Task 4)**
- ☐ Define/deploy optimization and target intervention possibilities (**Task 5**)

FY15 Goal – Study 4 - Candidate treatment courses

- ☐ Identify candidate treatment courses for GWI **(Task 6)**
- ☐ Select and test therapies in animals **(Task 7)**

FY16 Goal – Study 5 - Perform translational human clinical trials

- ☐ Verify treatment effectiveness in human subjects n=30 **(Task 8)**

APPENDICES

Spectral Analysis of Heart rate Variability - Using Echocardiographic Measurements

	Sedentary	Exercise
RRI (ms)	134.34 ± 6.30	142.37 ± 3.26
SD (ms)	4.96 ± 0.46	5.76 ± 0.44
RMSSD (ms)	3.29 ± 0.50	4.76 ± 0.79
LF abs (ms²)	1.46 ± 0.58	2.36 ± 0.42
HF abs (ms²)	3.12 ± 0.65	7.65 ± 2.25
LF (%)	21.00 ± 5.62	17.40 ± 1.79
HF (%)	55.25 ± 5.40	48.40 ± 7.17
LF (nu)	27.00 ± 6.29	29.00 ± 4.41
HF (nu)	73.00 ± 6.29	71.00 ± 4.41

Table 1: Spectral analysis of heart rate variability. Sedentary mice (n=5) and mice with exercise training (n=5) in wheel (5 days for week, during 2 weeks). R to R interval (RRI), mean standard deviation (SD), standard deviation of consecutive beats (RMSSD), sympathetic modulation of heart rate in absolute values, percentage and normalized units (LF abs, LF % and LF, respectively), parasympathetic modulation of heart rate in absolute values, percentage and normalized units (HF abs, HF % and HF, respectively).

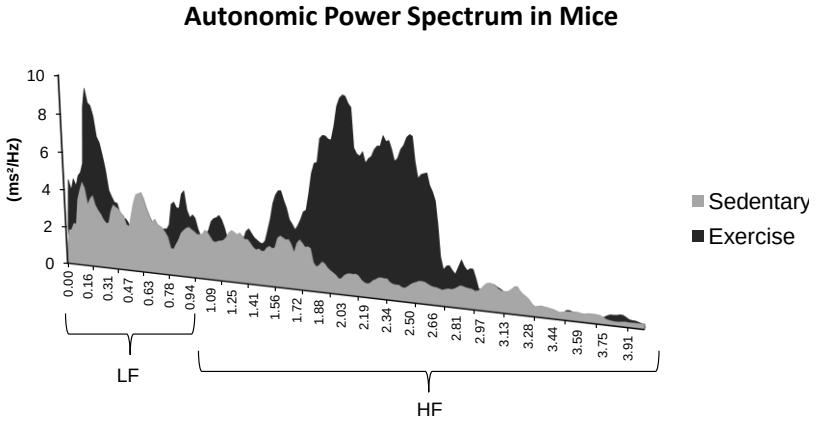


Figure 1: Autonomic power spectrum in mice. Comparison between power spectrum of sedentary and exercise trained mice. Low frequency band spectrum (LF – sympathetic modulation) and high frequency band spectrum (HF – parasympathetic modulation).

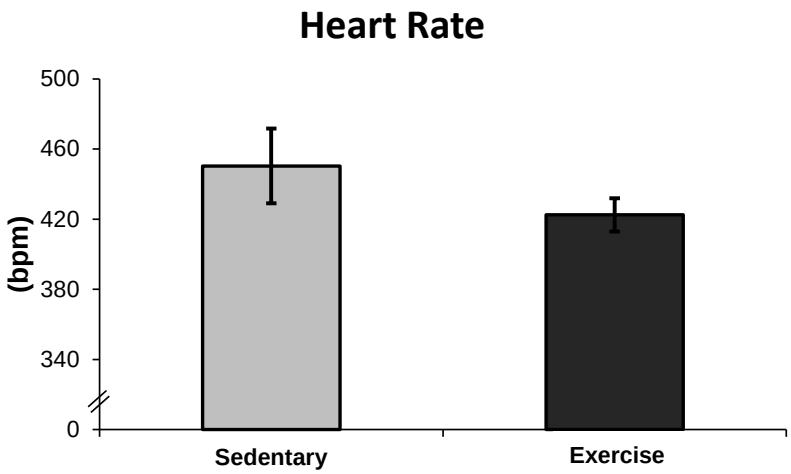


Figure 2: Heart rate of sedentary mice (n=5) and trained mice (n=5) after 2 weeks of wheel training (5 days per week, 1 hour per day).

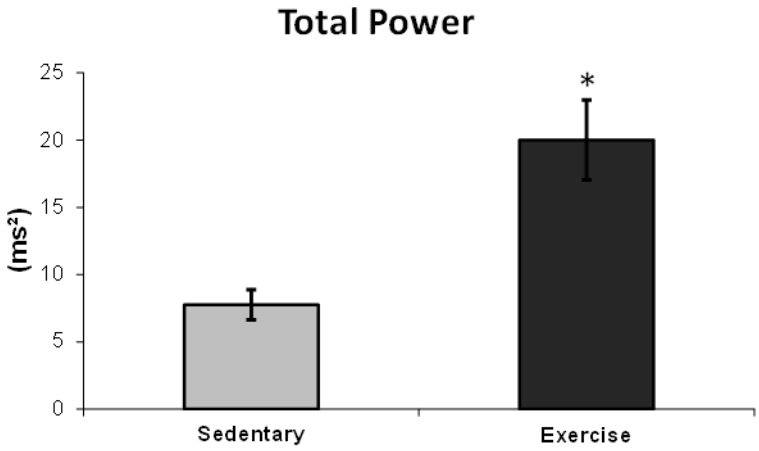


Figure 3: Total power. Heart rate variability in sedentary mice (n=5) and trained mice (n=5) after 2 weeks of wheel training (5 days per week, 1 hour per day).

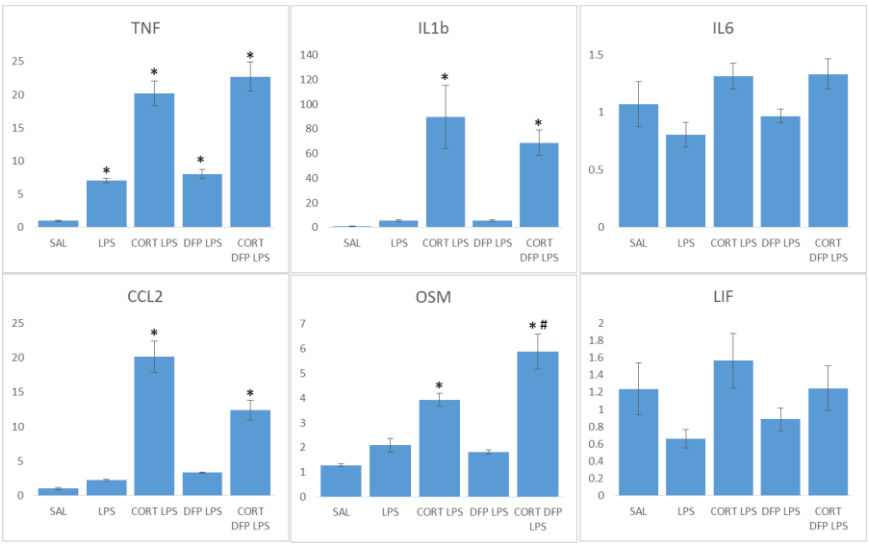


Figure 4: 21 Day GWI model - Results on cytokine expression in the cortex at 21 days following initial GW relevant exposure (4 days of Cort in drinking water, and single injection of DFP on day 5) that was followed up with administration of Cort in drinking water for 4 days every other week until 21 days. On this last day, mice were challenged with an inflammagen (LPS).

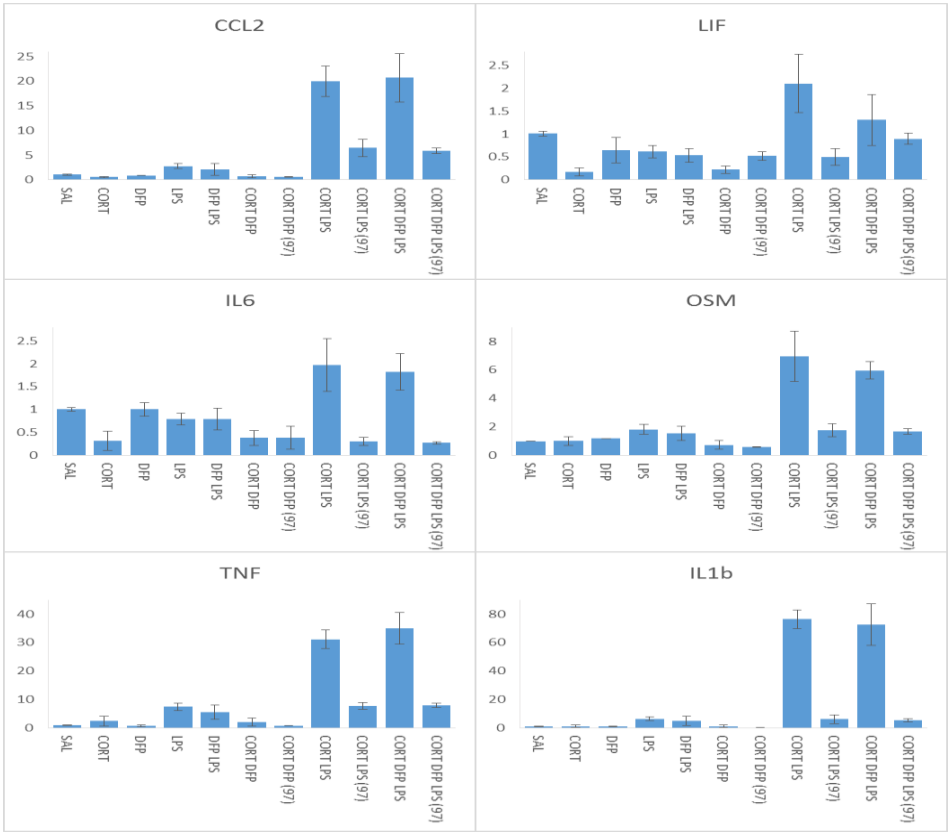


Figure 5: 90 and 97d GWI model - Results on cytokine expression in the cortex at 90 and

97 days following initial GW relevant exposure (4 days of Cort in drinking water, and single injection of DFP on day 5) that was followed up with administration of Cort in drinking water for 4 days every other week until either 90 or 97 days. On this last day, mice were challenged with an inflammagen (LPS).

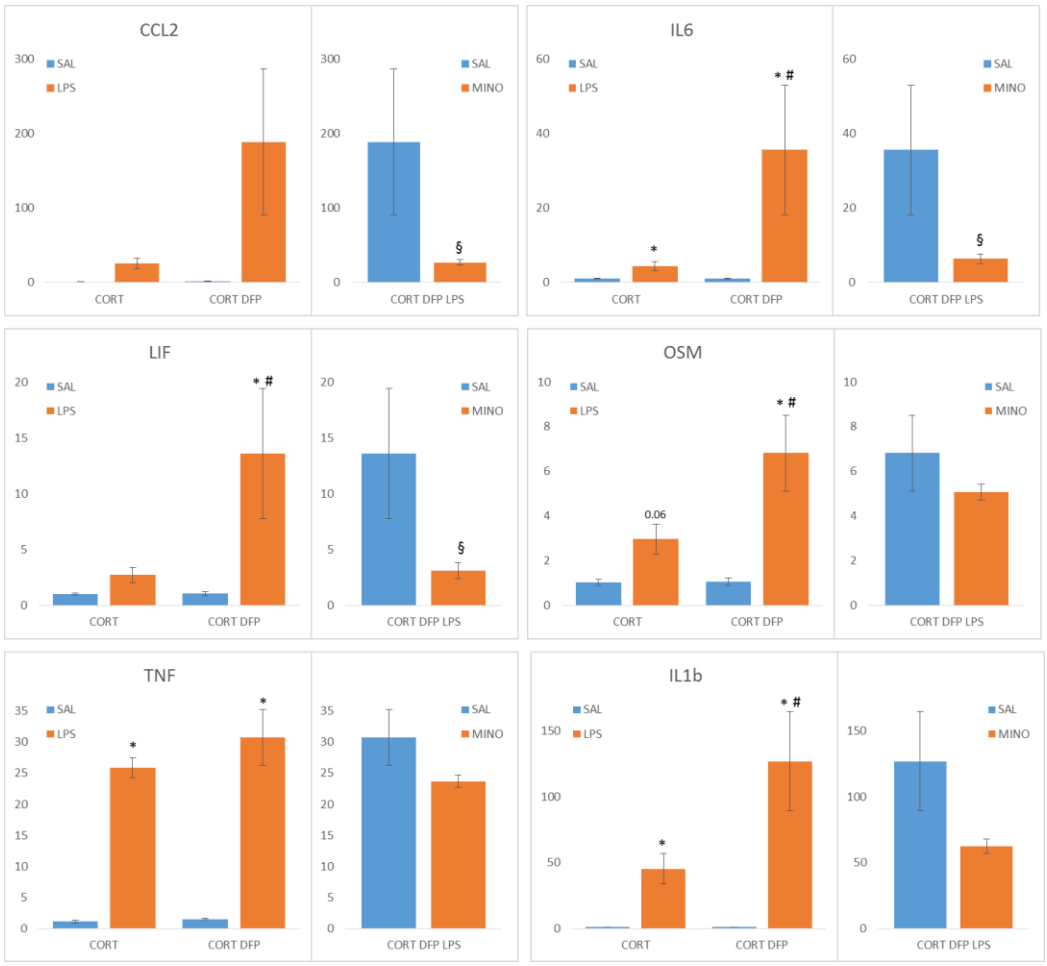


Figure 6: Mice were exposed to our initial GW theater-like conditions (4 days of Cort in drinking water, DFP on day 5) and then given an inflammagen challenge (LPS) 48 h later (on day 7). Mice received either saline or minocycline one hour prior to LPS exposure.